

B 210

OCT 15 1940

- Anti-Ea 3.87 II shown by precip. test not to have weakened in 5 yrs. 15 ml. dild. to 25 w. saline contg 1:10000 with. & by method again centrifg'd. from trace visat. ~~saline contg.~~

G.p. compl. (1:12.5 contg. 2 units in 0.1 ml (miss Bailey)) dild. w = vol. saline contg. 1:10000 with., chilled. centrifg'd.

~~dild. to 50 ml.~~ 5.0 ml. ea. res. diln.: + 5.0 saline + 4.0 saline + 1.0 Ea contg. 0.068 mg Ea N/ml., to remove in 25°A ; + 2.0 saline + 3.0 anti Ea soln. mixed, let stand in ice-H₂O.
Remove as 65% antb.

Set up:	1.0 ①	1.0 ①	1.0 ② Supnt	2.0 ③ Supnt	2.0 ③ Supnt
in test.	1.0 Compl. (1:1)	0.5	1.0	0.5	1.0 Ea .044
in ice-H ₂ O.	1.0 Saline + meth	1.0 Ea contg 0.053 N	1.0 Ea contg. 0.044	1.0 Ea contg. 0.044 N	0.1 partly antibl.
	<i>0.4 ml. antb. contains as compl. contg. in ② & ① removed no contg. as anticompl. by mistake</i>	<i>0.1 ml. antb. contains as anticompl. in ② & ① removed no contg. as anticompl. by mistake</i>			
	1.0 2 Supnt	1.0 ①	1.0 ①	Single 1.0 ①	
	1.0 Ea .044	1.0 Ea .053	0.5 i.c.	a 0.3 - 0.35 i.c.	
			1.0 Ea .053	.5 saline + 0.2 + compl. 0.15	
			0.5 partly anticompl.		

OCT 16 - Tubes centrifg'd., ③ tubes st. opalescent, centrifg'd. longer. washed down w. 4 drops chilled saline, homogenized, then w. 2 ml. more. 1st supnt - centrifg'd. & washes. added to residues from this. 3 washes. altogether.

(actual compl. diln.)
Compl. fl. @ $\frac{1}{20}$ gives complete hemolysis w. 0.2 antb. red cells. $\frac{1}{24}$ incomplete
 $\frac{1}{15}$ used to anticompl. test.

① " 0.67 should
have contg. 1 unit
∴ 4 contained 4 1 unit
not $\frac{7}{6}$ left.
(50 units
used.)

$\frac{0.6}{2} \text{ not } \frac{7}{6}$
110 units used
0.3 dilut.
0.2 .. not anticompl.
0.1, 0.2, 0.3 higher dilut.
contained w. antb.

For 2nd anticompl.
0.3 Supnt

OCT 24 - Anti Ea 3.87 III : 3 ml. dild. to 10.5, few drops P_H red added, neutral. w. $\frac{1}{10}$ HCl to pH 6.8.

Let fresh g.p. serum, $\frac{1}{3}$ from day before. $\frac{1}{2}$ inactivated.
Remainder chilled, carefully neutral., in stirring to pH 6.8 w. N HCl. Dried
tested w. saline + P_2O_5 ad. Inactd. compl. also inactivated. in air dry.

OCT 25 -

OCT 25 — Set up 1.0 diln. 3.8/III
 1.0 ser. 1.0 ser. 1.0 ser. 1.0 ser.
 1.0 Ea wthg 0.08 N 1.5 g.p.ser. 1.5 inact.g.p.s. 1.5 g.p.s. 1.5 g.p.s.
 1.0 Ea 1.0 Ea 1 salt 1 salt

After ca 1 hr. R. won temp. centrifg'd. in cold.

Titer: 0.1 undil. compl., 1:10 contains 2 hem. units. ∴ 1 ml. contains 200 units.
 1 Ea g.p.s + 1 Ea i.g.p.s supnt. unfortunately mixed.

Bf inputs - also run again, & all tubes washed 3x ^{C as bl.}
 2 i.c. supnt. contains no compl. Dild. 3:10, & 1:10 g.p.ser. 0.1, .2, 25¹ showed
 complete hemol. 0.03 undil. 0.3, undil. also.

Ea - ser. + cells + 0.1 compl. — hemol compl. wth 0.1, 0.5 wth 1, but 1 salt
 anticompl.

Ea - C - ser. supnt. 3:10, 0.2, 0.4 no hemol.

" " " " undil: 0.2, 0.3 no hemol.

" " " " " , 0.2, 0.3 + compl.: 0.2 hemol complete
 0.3 almost "

OCT 26 — Freshly dild. except. now contd. 1 unit in 0.12 ml.

1 " wthg compl. tube — no hemol. w. 0.3 - 0.6 inclusive.

" " C, Ea " — " " " "

" " " " " — hot anticompl. in 0.4

i.c Bf — 0.2 ant anticompl., 0.3, & higher anticompl.

OCT 29 — Anti P₁ III ab. ser 6062B. centrifg'd. 1/2 ml. dild. to 14, mactod @ 56°. ^{w saline - mactod, heated. P₁}
 Compl. — g.p.ser. from 3 pigs.

t = 18°. 1.0 ml. portions dild. ser. in all tubes.

1.0 " compl. in bls., 1.5 in >-ser. tubes. 1 saline also in bls.

1.5 " inactd compl in bls. x >-ser. " 1 saline in S-c + s-icbls

1.0 ml. S III wthg 0.04 ang. used in ^{all} S-ser tubes, as this leaves some A.
 Tubes washed 3x.

250. Titer: of compl. 1:10 diln. 0.03 - 0.08 + 4 saline + 1.0 wthg. cells.
 0.04 contains min. hem. dose. 4 ml. dild. to 5 w. 1
 would contain 2 units.

Compl - ser. - saline bl. wthg - 0.15 dild to 5 w. saline, 0.4, 0.5, 0.6, 0.7 set up w. 0.2 P₁.
 Hemol. complete in all, showing 0.06 w. + saline not anticompl. in dilns used.

375-
 84
 2nd
 370. Compl-ser. S supnt. 0.5 .1 .2 .3 .4 .5 .5 < 1 w. $\propto \frac{1.5}{3.5} \times .5 = .21$ compl
 saline .4 .3 .2 .1
 S-c .2 .2 .2 .2 .2

Hemolysis: none in any tube.
 No compl. in 1 " wthg. of C-ser-Ea.

2 units compl. added to ea.
 Complete hemol. in all tubes.

B 210 , Ppts. in pres. of compl.

OCT 25 - Sols. 204A, which had been standing in cold in cotton-plugged bottle, centrifg'd. cold from sl. sediment, inacted. @ 60° for hr., chilled overn. 26" - Centrifg'd. Supnt. found barely anticompl. C 1:5 by Mrs. Lipman. Accordingly following set up in cold 2.0 ml. 204A + 7.0 ml. saline contg 1:20000 methyl & 15g. p. serum (compl. action evident only undil.).

Same, + 0.2 mg. S III

2.0 ml. 204A + 8 same saline, + 0.2 mg. S III.

5.16 mg. mit. N/ml.

Nov. - Ca. 1.8 ml. Ser. 4.75, dild. w. ca 6 ml. saline, set up as follows w. g. p. complement from Miss Baker, Wassermann Lab. same day, 20 units/ml. Bls.: 1.0 ml. x 1 4.75 dild. + 1.7 saline

Dupl. 1.0 ml. portions 4.75 dild. w. 0.7 compl.; 1.7 saline + 0.15 SI

S made up in Oct. 1/23-4 in 0.15 ml.; 1 saline + 0.7 compl. + 0.15 SI, all at 0°.

2" - Supnts + w. S 2" - Pts. mixed 2x, but as settled rapidly, centrifg'd. cold, S should have been added w. calibr. pip. washq. w. 3 or 2 1/2 ml. chilled saline.

3" - 5 ml. same ser. dild. thrice w. 15 saline, inacted.

4" - Tests on supnts. from Nov. 2 w. sensitized cells + compl. 1:10 from Miss Pauli 475, saline - not anticompl; no compl. up to 0.4 ml. 0.1 + 0.2 compl. lyed 0.2 + 0.25. " " G.P. No compl. left. in 0.05, 0.1, 0.2 sl. in 0.4?

5" - Dils. of Ser. 475 5:15 (Nov. 3, inacted.) used. This + compl. centrifg'd. cold. Compl. active 1:10, 0.1 ml., test at beginning of expt.

Set up at room temp.: 25-6° Tubes in dupl.

	1	2	3	4	5	6	mixed
475 dild.	1.0	1.0	1.0	1.0	1.0	0	
GP ser. (pooled, 2 animals w. strep. adenitis) 7.0 ml.	0	1.0	0	1.0	1.5	1.5	reg.
Saline	1.5	0.5	1.5	0.5	0.5	1.5	
S	0	0	0.2	0.2	0.2	0.2	

Centrifg'd. room temp. Dupl. supnt. combined. Pts washed 2x w. saline @ room temp. No whirl in Ser. 1, 2, 6. Bl. pts. - considered neg.

0.1 on repetition hemol.

Rds after 15 min. Supnts: 1 - 0.2 sl. anticompl. 2 - .025 + 0.05 compl.
2 .03, .04 hemol. complete 4 - 0.1, .2 no hemol 5 - .10, .2 ±
2 0.6 - - 4 - 0.4, 0.6 " " 5 - 0.4, 0.6 0
9 in absence of other controls, ast > 1/20 orig. compl. present in (4), (5).
5 - 1" wash. 0.05, 0 0.2 0 0.4 0 0.35 not anticompl.

B 210

Nov. 10 - 1.0 ml. portions same 4.75 diln. centrifg'd., set up w.
following in dupl.; 1.0 fresh compl. from Miss Baker (last titr. ~~2.0~~ units per
0.1 ml.) (mine, ~~2.0~~), 0.25 mg. in 0.25 ml. S 123-4 date of Oct 1, '37
; 1.0 ml. same compl., heat inactd., 0.25 S; 1.0 ml. old compl. from Mrs. Lipman
now only 0.8 unit in 0.1 ml.), 0.25 S, 0.5 4.75 diln., 1.0 old compl., heat inactd.,
0.125 S. + 0.5 saline @ room temp. $^{22^{\circ}}$ P.v. settled quite rapidly. 1 drop
tween added to ea. & covered w. card.

11" - P.M. 0.1 good compl. diln. still hemolyzed. .05 also
Supmt., 1.0 ml. ^{good} new compl.: no hemol. in 0.1, 0.4, 0.6
" 1 ml. old " 0.2, 0.4, 0.6

Soln. 205 D' 0.46 mg. anti. N/ml, 0.05 SIII being low not.

23" - Portion w/ aer. mist. 556 + 633 inactd. after centrifg'd. Centrifg'd.
again. On basis of prelim. tests., all at vol. 3.0 ml., warming to 37° , 0, 4, 8, 12, 16,
20 units compl. added to 0.50 ser., mixed, to all but ser.-compl. & (15)
1.0 ml. SIII soln. contg. 0.07 mg. S added. Let stand ca 37°
for 15 min. (flocked after few moments), w. occas. mixing. Centrifg'd.

In 4 ml. vol., 0.2 sensitized cells hemolyzed ^{completely} in 15 min. @
 $38-40^{\circ}$ by 4 unit compl., in ca 40 min. by 2 units. If 0.5 ml.
above ser. present, 2 units showed consid. inhibition in 15 min. but comple
hemol. complete in ca 40.

4 ml. dupl. of 8 + 20 set up w. 0.2 sensitized cells. At end of
15 min. little hemol. noticeable, but after ca 40 min. hemol. partial
in 8, almost comple in 20. ∴ ca 1.5 units in 8, 2 in 20.

Nov. 29 - 5 ml. 4.75 diln. to 25 w. saline, inactd. 15 more ml.
saline, contg 0.15 ml. serum added. Compl. from Miss Baker, said to be
2 units in 0.1 ml. 1:10, but on test reg. 0.15 ml. 1:10 for 15 min. hemol.
@ $37-40^{\circ}$. This called 2 units. Bl 4.75 diln + saline + 1.5 ml.
1:10 compl. diln. set up (20 units). Also 0, 4, 12, 20, 50 units compl.,
last being 0.375 ml. freshly centrifg'd. undil. compl. All made up to
2.3-2.5 ml. w. saline, warmed to 37° , 0.125 ml. SII, 1:1000 added to ea.,
mixed, let stand 20 min., w. occas. mixing. Centrifg'd. in C centrifg.,
0.15 ml. of 20, 50 supmt. did not cause hemol., zw. 0.6

Entire remainder of 50 caused ^{very} incompl. hemol., 20 Bl. compl.
∴ Supmt. of 50 contained not > 2 units compl. 0 supmt. + aer. - , + S II

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NOV. 5 - 2.0 ml. same 6-062f titld - w. 12 saline - with, inactrd. @ 56°,
 Serum taken from 3 large g. ps.
 6 = Compl. ser. configd. compl. neutral to Ca^{+2} 7.4 w. N HCl . 45 ml.
 1/2 inactrd. 4.0 ml. compl. Bls. + 0.8 ser., + 0.03 S III.
 1.0 ml. ser. Bl.
 $t = 23^\circ$
Duplicate 1.0 ml. ser. + 5.0 compl. + 1.0 S III - with + 0.045.
 1.0 " " + 5.0 inactrd. " + " " "
 Inactrd. compl. Bls. : 3.0 ml. + 0.6 ser., 0.024 S III. Also: 1.0 ser.
 i.c. tube flockcd before c tube. + 1.0 S III sol.
 in trip.

Compl. titres.: 0.1 ml. made up to 10. 0.3 - 0.7 set up w. 0.2 mixed red cells. .3 almost fully hemag., 0.4 ^{0.7} compl. ∴ 250 titres. 4./ml.

Coupl. res - Bl. titm: 0.12 made up to 10, 0.4 - 2. Complete remvt,
showing no anticompl. action of 606 res present.
0.06 undil. gave complete remvt.

Compt. - ser. - 5. sulphab. 0.05 v.l. no hemol.
 0.1 - 0.7 : no hemol. 2 u. 7 ml. added to e. : [Compt. Compt. -
 in all.]
 Inacted. Compt. - ser. - 5. sulphab. 0.3 - 0.7 + 2 u. ^(1.1 - 1.2) Compt. - ser. Vt. 1:10
 no hemol in any! 0.2 serial. cells hemol.

2 vols. on rainfall.

0.2 ml. re utilized red cells. Count made by Mrs. Smith on 3 successive samples. 1,590,000, 1,470,000, 1,380,000 per cu. mm.; last probably is over.

Ea - anti Ea 3.87 II 15:25

0.1 made up to 10 w. saline. 0.24 portions. cont.

0.3 ml. 1:10 above C-125 bl. with 6 cu. compl. ^{titrated}

0.0011 Ea N/ml., 0.2 should be ok. John made up 0.0044 Ea N/ml.

0.05 to .3 set up. w. .2 ser. diln. after addn. of 6 u. wmp.

Incub. in 1/2 hr. at 37°, then 0.2 ¹ ml. red cells added.

9 m 10 min @ 37° 1 tube + + K-mol. .1 + .15 mol (amt.), .2, .3 complete.

" 20 " " 37 05 444 .14 " " " " " "

Repetition of above w. 2 u. compl. gave complete fixation bet 0.25-3.4

B 210

Nov 11 - 2.0 ml. 6.06% dild. to 20 w. saline - mostly, inactd. chilled,
 orig ca^{6%}. filtered through collodion disk filter (700 µ) vented to Ph ad. (2 drops in cu).
 After 5 min. 7.3 u.
 10 drops N
 HCl.

12" - 52 ml. compl. 22 inactd., centrifg'd. cold.

4 5.0 ml. portions, ³w. 1.0 dild. ser., ~~3~~ 1.0 ml SIII contg.
 SIII, 1 w. ~~compl.~~, 0.5 SIII soln. Last centrifg'd. w. others,
 supr. centrifg'd. discarded

compl. Bl. 1: 5.0 compl., 1.0 ser., 1 saline into fresh tube, 0.50
 1: 4.5 " 0.9 " 0.9 " ^{supr. centrifg'd. discarded}
 1 ml. ^{ser. diln. added,} ^{at stand. temp.,}
 3 5.0 ml. portions i.c. + 1.0 SIII soln. ^{centrifg'd.}

In compl. Bl.: 1 5.0 ml. + 1.0 ser. + 1 ml. + ~~0.50~~ ^{1 ml.} SIII soln.
 Difl. ser - 4 ml. saline &c.

Implicate 1.0 ser. - 4 ml. saline - 1.0 SIII soln.

Tubes without q.p. ser. v. i.c. tubes flocced more coarsely than compl. tubes

Compl. titrn: 1:100 1" tenu bet 0.4 & 0.5

2" tenu (1 w.) 0.3++ 0.4 compl. 0.45 compl. 0.5 - .6 compl

Compl. ser. bl. 0.15 to 10 ad.: 0.3++ 0.4 presch. " " "

0.2 ml. sensitized red cells: only 980,000, 990,000, 965,000 per cu. mm.

Compl. - ser - S supr.: 05, .1, .3, .5, .7 almost complete
 1 u in 2 " " .1 .15 .2 .25 .3 .35 hemol. 3 less on
 35 u left. almost complete hemol. 2, less on either side.

= 1" wshg. " " no hemol. 0.1 - 0.7 complete hemol. after adding 1/4 u compl.

200 u 1/2 S ser: complete hemol. 05 supr. 1:10 set up .1 - .4
 left. 0.3 1" tube completely tenu.

0.2 sensitized red cells + serum + 1:10 rabbit ser. 05 .1 .15 .20 .25
 Compl.

NOV 13 - C-Bl-sup. now requires 0.3 1:50 for complete hemol.
 200 u 1/2 S ser - S sup. 0.15 almost complete hemol. .2 u, complete. Repeated, starting at 05:

= 40 | 80 u 2" wshg. : no hemol. 0.2 - .7 1 u. compl. added. no hemol.
 u " 1" wshg. 1/2 S-m-C: no hemol. 1, 3, 5, 7, 1/3 u. " " . Complete " .

Rabbit plasma 1:10, no hemol. in 0.05 - 0.2

Dec 3 Cp ser Bl up = still compl. hemol. w. 0.02 & 0.05 undil.
 9 Cp u u u no hemol. 0.1, 0.3.

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NOV 15 1940

(1:1)

MC 131940. — "5%" sheep cell susp. gave 640,000, 630, 650 per cu mm.
 $12\frac{1}{2}$ centifg. in cold. cell vol. 4%. Made up to 8. Count now 820, 815, 800
 instead of expected millions. Centifg. again. Cells taken up to 7 ml.
 0.50 ml. cells + 1.0 saline; 0.50 ml. diln. for Kj. Inpt.
 2.50 ml. Kj. / H6 / 40 cu mm. for

All 3 sets 2 cont. snap. + 1.33 1:1250 sensitized set.

Made up to 2 ml. susp. + 2 sensitizing diln. " " " .08 hemolytic

0.6 w. saline
before reading.

Count 2 ml. msp. #3 sterilized dish.

Nov 16 - Same 1:1 serial. susp. set up w. fresh 1:50 diln. same comp.
^{0.75}
+ ++ +++ complete Also 1:1.5 vol unit. - sp. : 1 ml. + 0.2 saline
+ ++ +++ "
 \leftarrow to give same amt. crit. on fewer cells.
 \therefore Unit in ~~#~~ expt. of Nov. 12 was not < that in
preceding expt., although no. of red cells in hem. unit was small.

- ① 0.25 undil. compl. + 0.05 173 15:25 3.87 II delay.
 ② " " " + 0.01 HS 902 (~know.)

Remainder of 902 treated w. 1% wet 15 minutes, entombed
0.06 to 1.5

0.2 1:50 diln. ① gave ++ const.; 0.25 complete. 0.2 1:50 " ② " ++ " ; 0.25 " . weakens
going to 1@2.t.

Rab. A 19, B 31 sec. Food overall on plot, 1:10
.1, .4 No haul. .1 ±, .4 ++±

*Human, James Mitchell, moderate ulcer, size drawn on 14"; 1:10.
-1 ++ , -4 complete

wshgs. of g.p. clot, ca 5 ml.
1/50 3 +++ 4 +++++

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NOV 18 1940 - 8 ml. 3.87% 15:25, neutralized, diluted w. 16 ml. saline nutrit., again centrifuged. Neutralized w. $\frac{1}{2}$ HCl to ca pH 6.2. Centrifuged.

402 sec. also neutralized, centrifuged.

65 ml. g.p. 2-7, neutralized, again centrifuged in 6 nutrit. in 11 cups, run through 3 of same collodion filters.

NOV 19 - 10 ml. 5% sheep cell susp + 10 1.1250 hemolysis (from Wassermann) 2nd. w. above g.p. compl. 1:100 \downarrow Dif = 775, 80; 840, 000; 940, 000 $\frac{\text{Labs}}{\text{cells/cm}^2}$

11 AM	0.2	.25	.3	.35	.4	.45	.50
					++	+++	complete

Repetition "

:

++ + ++ + +++ "

Compl. 1/50 Rins " 1/10 ++

Complete

Complete

$\frac{1}{5}$ Red cell susp. 1/1 dilution

Hum. sec. " .03 + net. Complete .04 + net. Complete .06 + net. Complete.

" " 1/10 .3 Complete .25 almost .

Horse's Day 402	1.0	1.0	1.0	1.0
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Salt 30

30

30

30

IC

C

Mixed.

ST, 0.5 mg/ml.	1.0	1.0	1.0	1.0
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Let stand 1 hrs. @ room temp. 20° centrifuged cold.

Supnts. centrifuged again. Ppts. w. washed 3x w. 10 ml. chilled saline, pl. also. supnts. re-centrifuged. 20° - Ppts dried in alls., made up to 20.0, 6.0 ml. for anal. in dupl.

19" - ^{supnt.}

5.5 ml. I.C. in all tubes: 1.0 3.87% 1/3, 1.0 E nutrit 0.036 mg N $\left\{ \begin{array}{l} 1 \text{ sec.} \\ 1 \text{ Ea.} \\ 1 \text{ R.} \\ \text{conty.} \\ .50 \text{ Ea. N} \end{array} \right\}$

" " C " " " " " " " "

4.5 hum. sec. " " " " " " " "

5.0 g.p. clot wshgs. " " " " " " " "

1.5 orig C, 0.05 " S I sec. No visible ppt.

Titn. of Remaining portion of C supnt. : 0.11 ml. up to 5 = 1/50

.2	.225	.25	.275	.3	.35
				++	+++

Few comp.

Hum. Opt. rev @ 45°

0.50 C sup. add. to 5 w. saline 1.1 to 5 to give 1:50

25/32 / 6.0	25/32 / 6.0 / 1.0
	225

150

